Aryl hydrocarbon receptor (AhR) regulates adipocyte differentiation by assembling CRL4B ubiquitin ligase to target PPARγ for proteasomal degradation

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List of the supporting information:

Table S1. Primers used in real-time PCR.

AhR fwd:	5’-GCCAGGACCAGTGTAGAGC-3’
AhR rev:	5’- ATTCAGCGCCTGTAACAAGAA -3’
PPARγ fwd:	5’- CCATTCTGGCCCACCAAC -3’
PPARγ rev:	5’- AATGCGAGTGGTCTTCCATCA -3’
CUL4B fwd:	5’- ACCTCGTCCTTCTGCCTTGGG -3’
CUL4B rev:	5’- CTGAGCCGGGCTTGCTGTTAG -3’
GAPDH fwd:	5’- TGTGTCCGTCGTGGATCTGA -3’
GAPDH rev:	5’- CCTGCTTCACCACCTTCTTGAT-3’
Fabp4 fwd:	5’- GATGCCTTTGTGGGAACCTG -3’
Fabp4 rev:	5’- TCCTGTCGTCTGCGGTGATT -3’
Adipsin fwd:	5’- CTGAACCCTACAAGCGAT -3’
Adipsin rev:	5’- GACCCAACGAGGCATTCT -3’
CD36 fwd:	5’- GGCCAACGTATTGCGACAT -3’
CD36 rev:	5’- CAGATCCGAACACAGCGTAGA -3’

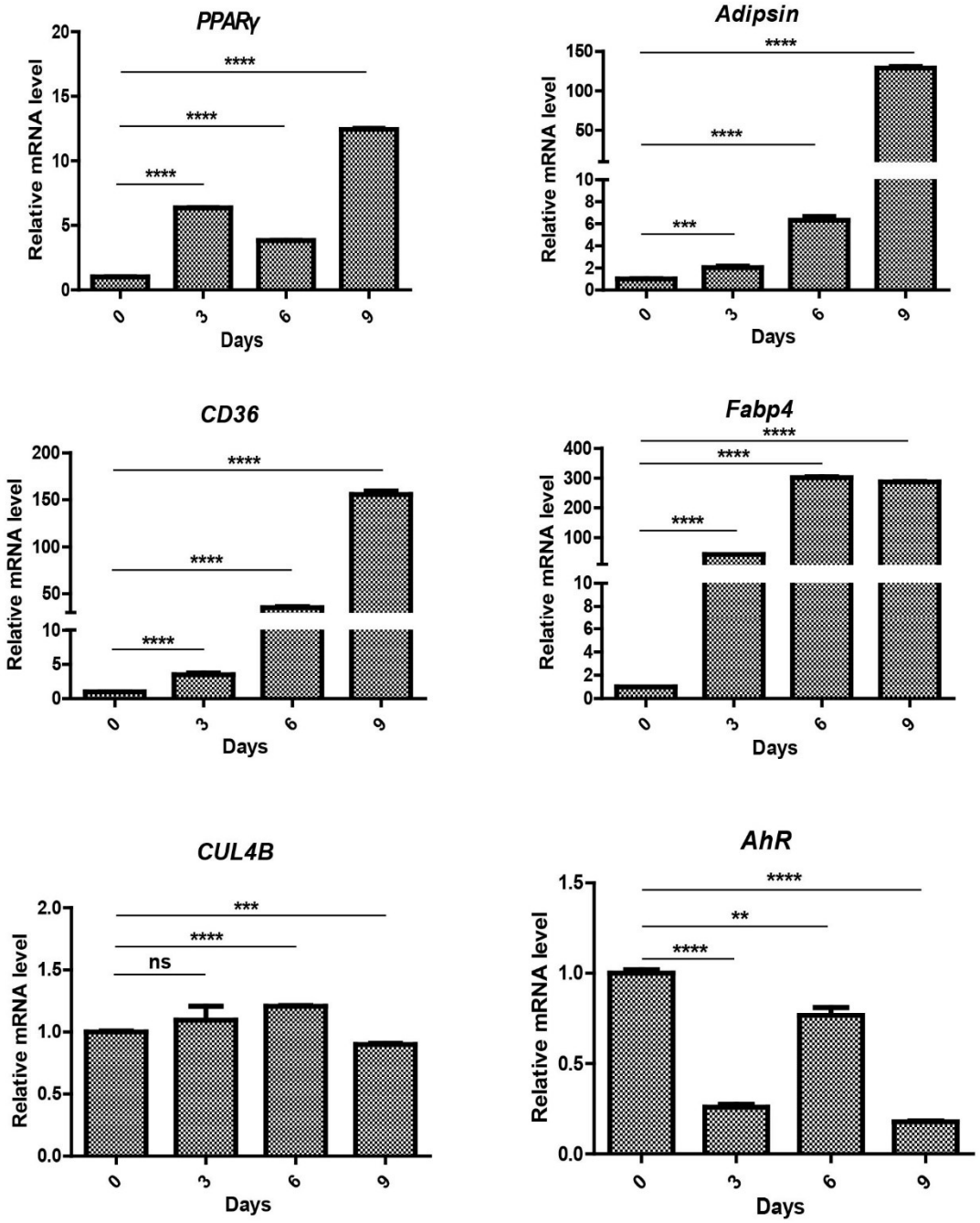

Figure S1. 3T3-L1 differentiation. mRNA levels of *PPARγ* and its related genes in 3T3-L1 cells. At different time points post-treatment, total RNA samples were extracted and subjected to qRT-PCR analysis of related genes (*Fabp4*, *CD36*, *Adipsin, Cul4b* and *Ahr*). Data were presented as mean ± SD.; n=4 with $^{**}P < 0.01$, $^{***}P < 0.001$ by student's *t* test.

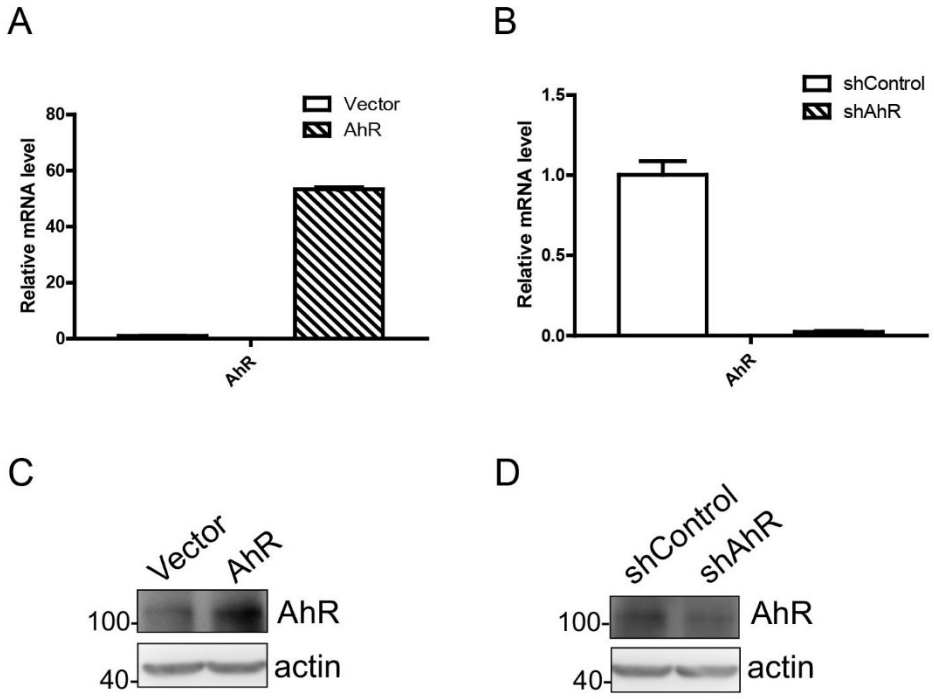

Figure S2. Efficiency of the overexpression and knockdown of AhR in 3T3-L1 cells.

A and B, The mRNA levels of overexpression and knockdown of AhR were validated by qPCR.

C and D, The protein levels of overexpression and knockdown of AhR were validated by Western blots.

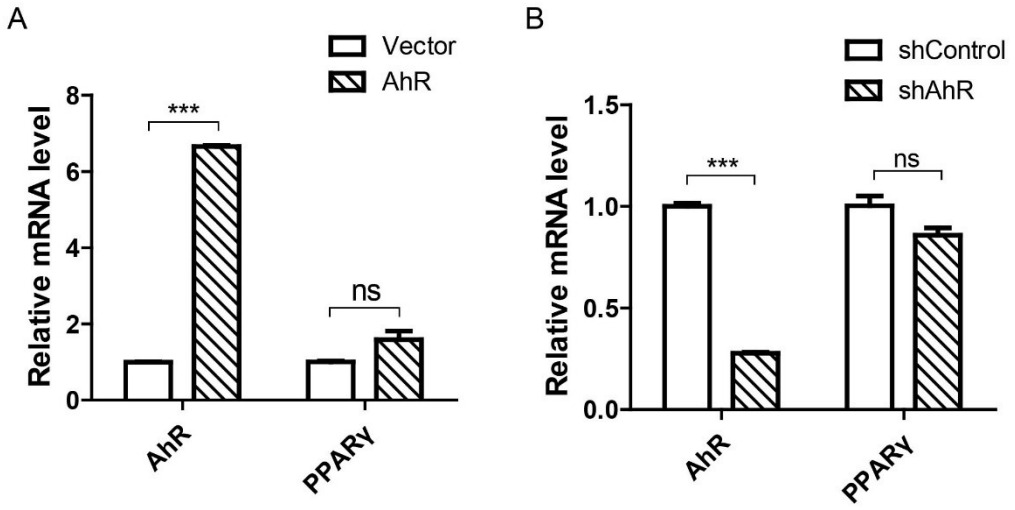

Figure S3. AhR did not affect the transcriptional level of PPARγ.

A, AhR did not affect PPARγ mRNA level in wild-type and AhR overexpression 3T3-L1 cells. mRNA levels were measured by qRT-PCR analysis using primers targeted for AhR and PPARγ.

B, AhR did not affect PPARγ mRNA level in wild-type and AhR knockdown 3T3-L1 cells. mRNA levels were measured by qRT-PCR analysis using primers targeted for AhR and PPARγ.

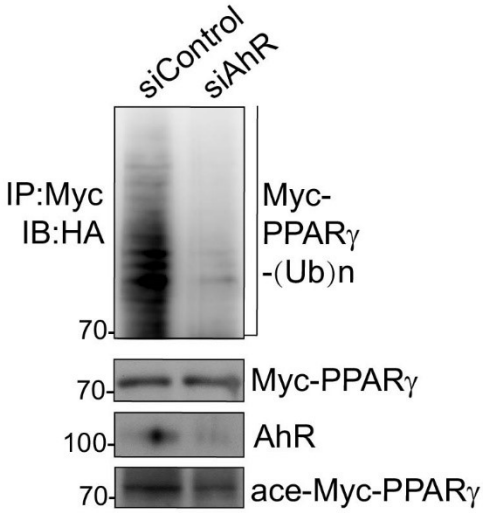

Figure S4. Defect in ubiquitination does not increase acetylation level of PPARγ.
3T3-L1 cells were first transfected with siAhR or siControl. Cells were then transfected with plasmids expressing Flag-Cul4B, HA-Ub and Myc-PPARγ. Cells were treated with MG132 for 3 hours. Cells were then harvested for the following immunoprecipitation and Western blotting. Input (5%) was used for Western blotting.